

Engineered PEG Hydrogels with Enhanced Proteolytic Degradation for Presentation of Angiogenic Signals

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Statement of Purpose: Hydrogels formed from Michael-type addition reactions of end-functionalized poly(ethylene glycol) (PEG) macromers with thiol-containing protease-sensitive peptide crosslinkers have previously been described as matrices for cell-induced enzymatic remodeling [1]. To impart bioactivity, the modified multi-arm PEG can first be functionalized with cysteine-containing cell adhesion ligands, growth factor binding ligands, or growth factors before crosslinking to form the hydrogel network. Previous research has shown that physiologically normal angiogenesis can be induced by sustained delivery of vascular endothelial growth factor (VEGF) from these hydrogels [2]. However, for supporting angiogenic sprouting into the hydrogels, their remodeling rate may be too slow and may limit cellular infiltration. Recently, we have shown that by modifying the protease-sensitive substrate within the peptide crosslinker, the hydrogels can be rendered more degradable, leading to enhanced cellular invasion and proliferation within the hydrogels [3,4]. In the present study, a peptide ligand shown to bind VEGF, SY_{SO3}DY_{SO3} [5], is incorporated into the PEG hydrogels to enhance retention of this angiogenic molecule and to show the combined effect of enhanced degradation and presentation of an angiogenic signal within the hydrogel in an *ex vivo* cell invasion/angiogenic sprouting assay.

Methods: Hydrogel Fabrication: Peptides were prepared by standard solid phase synthesis. Branched 4-arm PEG was modified and hydrogels were formed as previously described [1].

Release Studies: The VEGF-binding ligand (Ac-GCRDGSYSY_{SO3}DY_{SO3}G-NH₂) was reacted with the modified PEG and then incubated with VEGF prior to crosslinking to form the hydrogel network. Hydrogel samples were placed in a release buffer, and VEGF release was measured by an ELISA assay.

Chick Aortic Ring Assay: Segments of embryonic (day 8-10) chick aortae were embedded in the hydrogels and cultured in serum-free medium. Cell invasion over time was visualized by brightfield microscopy. At the end of the experiment, the aortic rings were fixed, permeabilized, and stained with Alexa Fluor 488-phalloidin, rhodamine-*Lens culinaris* agglutinin, and Hoechst 33342.

Results: Release experiments demonstrated that VEGF could be retained within molecularly engineered PEG hydrogels containing the SY_{SO3}DY_{SO3} VEGF-binding ligand (Figure 1). Previously, 29 peptide substrates were screened for degradation by MMP-1, MMP-2, and plasmin, resulting in a range of k_{cat} values for degradation of soluble peptides and a range of hydrogel degradation times [3,4]. Here, Ac-GCRD-VPMSMRGG-DRCG-NH₂ was selected as a fast degrading protease substrate crosslinker as it showed enhanced degradation by all three enzymes, and Ac-GCRE-GPQGIWGG-ERCG-NH₂ was used as a slower degrading crosslinker. In a chick aortic ring outgrowth assay conducted in serum free medium (so

that the only growth factors available were those presented within the engineered hydrogels), extensive sprouting from the aortic ring segments only occurred in the faster degrading hydrogels that also presented VEGF with its binding ligand, SY_{SO3}DY_{SO3} (Figure 2). All hydrogels also contained the cell adhesion ligand, RGD. Studies exploring additional protease substrates as well as different doses of VEGF are ongoing.

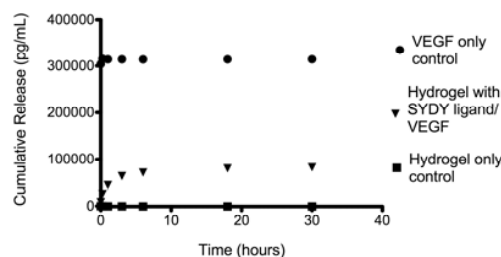


Figure 1. Release of VEGF from PEG hydrogels with the SY_{SO3}DY_{SO3} ligand and VEGF compared to VEGF with no hydrogel and hydrogel with no VEGF controls.

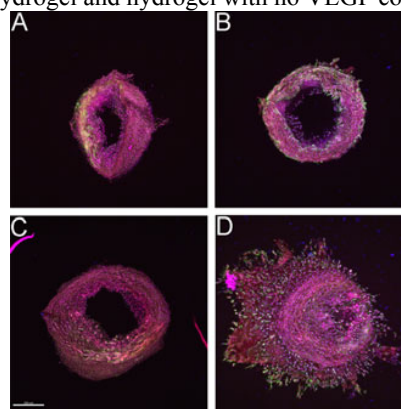


Figure 2. Confocal image stacks of chick aortic ring assay (scale bar = 200 μ m). (A) Slow degrading, no VEGF. (B) Slow degrading, SY_{SO3}DY_{SO3}, VEGF. (C) Fast degrading, no VEGF. (D) Fast degrading, SY_{SO3}DY_{SO3}, VEGF.

Conclusions: A sulfated peptide known to bind VEGF was utilized to incorporate this angiogenic molecule into molecularly engineered PEG hydrogels. In addition, protease sensitive peptides with increased k_{cat} values compared to the MMP cleavage sequence in type I collagen were used to render the PEG hydrogels more degradable. Only those hydrogels that were faster degrading and that contained an angiogenic signal were able to support cell invasion in a chick aortic ring outgrowth assay. These faster degrading angiogenic hydrogels have potential as matrices to support angiogenesis and tissue regeneration *in vivo*.

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